FEULGEN CYTOPHOTOMETRIC DETERMINATION OF NUCLEAR DNA IN SPECIES OF *NITELLA* (CHAROPHYTA) FROM INDIA

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ABSTRACT. – Feulgen cytophotometric DNA determination have been done in five species of *Nitella*, viz. *N. hyalina* var. and f. *hyalina* (n = 8), *N. furcata* subsp. *furcata* var. and f. *furcata* (n = 18), *N. stuartii* (n = 15), *N. furcata* subsp. *flagelliformis* (n = 9) and *N. acuminata* var. and f. *acuminata* (n = 18). Together with the DNA content, the nuclear volume and the chromatin length of the species have also been calculated. A direct relationship between nuclear volume, chromatin length and DNA content was found. The highest DNA content was noted in case of *N. hyalina*, whereas the lowest content was found in *N. furcata* subsp. *flagelliformis*.

RÉSUMÉ. – La méthode de cytophotométrie utilisée sur les noyaux colorés par la réaction de Feulgen a permis d'évaluer les teneurs en ADN de cinq espèces de *Nitella* : *N. hyalina* var. et f. *hyalina* (n = 8), *N. furcata* subsp. *furcata* et f. *furcata* (n = 18), *N. stuartii* (n = 15), *N. furcata* subsp. *flagelliformis* (n = 9) et *N. acuminata* var. et f. *acuminata* (n = 18). De plus, pour chaque espèce, le volume nucléaire et la longueur de la chromatine ont été calculés. Il existe une relation étroite entre le volume nucléaire, la longueur de la chromatine et la teneur en ADN. *N. hyalina* présente la teneur la plus élevée en ADN, tandis que *N. furcata* subsp. *flagelliformis* offre la plus faible. (traduit par la rédaction).

KEY WORDS : Feulgen cytophotometry, DNA, *Nitella*, Charophyta.

INTRODUCTION

Microspectrophotometric DNA determination have been in practice in plant materials for quite sometimes, yet very few attempts have been taken in case of algal genera, such as *Batrachospermum, Eudorina, Coleochaete, Acrosymphyton* and in Dinoflagellates. In lower groups, cytophotometric DNA-determination helps in studying the life cycle pattern and in locating the site of meiosis where the chromosome count is not possible (THERRIEN, 1966; BRYANT and HOWARD, 1969; HURDELBRINK and SCHWANTES, 1972; YEMMA and THERRIEN, 1972; LEE and KEMP, 1975; HOPKINS and McBRIDE, 1976; BREE-

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Source : MNHN, Paris

MAN, 1979; GRAVILA et al., 1981). In the algal family Characeae only a solitary report is available regarding the DNA determination in *Chara zeylanica* (SHEN, 1967).

In view of the paucity of information in this regard in any other taxa of Charophytes, the present investigation was taken up in the genus *Nitella*, where no such data are available so far. Principal emphasis was laid upon measuring the DNA content of mitotic figures. Such data is expected to throw some light regarding the mode of evolution of various intraspecific taxa and their relationships especially when taken in conjunction with data on nuclear volume and chromatin length of each taxon.

MATERIALS AND METHODS

Five species of *Nitella* were taken for the present study viz. *N. hyalina* (D.C.) Ag. var. and f. *hyalina*, *N. furcata* (Roxb. ex Bruz.) Ag. subsp. *furcata* var. and f. *furcata*, *N. stuartii* A. Br., *N. furcata* (Roxb. ex Bruz.) Ag. subsp. *flagelliformis* and *N. acuminata* A. Br. ex Wallm. var. and f. *acuminata*. Specimens identified after WOOD and IMAHORI (1964, 1965).

Young vigorously growing shoot tips with suitable sized globules were fixed in formaldehyde acetic-ethanol (FAE) fixative for one hour. They were washed and hydrolysed in 5 (N) HCl at room temperature for one hour, again washed and stained in Feulgen solution (LILLIE, 1951). After staining, the materials were bleached by bleaching solution (60 ml (N) HCl, 60 ml 10 % aqueous solution of sodium or potassium metabisulphite and 1080 ml distilled water) with two changes of 5 minutes each to remove the superficial stain, washed and finally mounted in 10 % glycerine. Cytophotometric readings were taken in Reichert Zetopan microspectrophotometer within two hours of making temporary slides. Readings were taken from the metaphase stage of the antheridial filaments of the globules to determine the 2C DNA value. The measurement of DNA was based on the optical density in terms of arbitrary units of relative absorbances (SHARMA and SHARMA, 1980).

Total chromatin length was expressed by adding the lengths of all the chromosomes of a metaphase plate. Average of five such plates were taken to calculate the final value. The nuclear volumes were calculated according to the following formula :

$$\text{Nuclear volume} = 4/3\ \pi\ (r)^3$$

where, r = radius of the nuclei

RESULTS AND DISCUSSION

Among the five species of *Nitella* studied, *N. hyalina*, one of the two heteroclemous taxon investigated showed the highest 2C DNA (Table I; Figs. 1 & 2). Excepting *N. furcata* subsp. *flagelliformis* a dioecious species whose chromo-

Source : MNHN, Paris

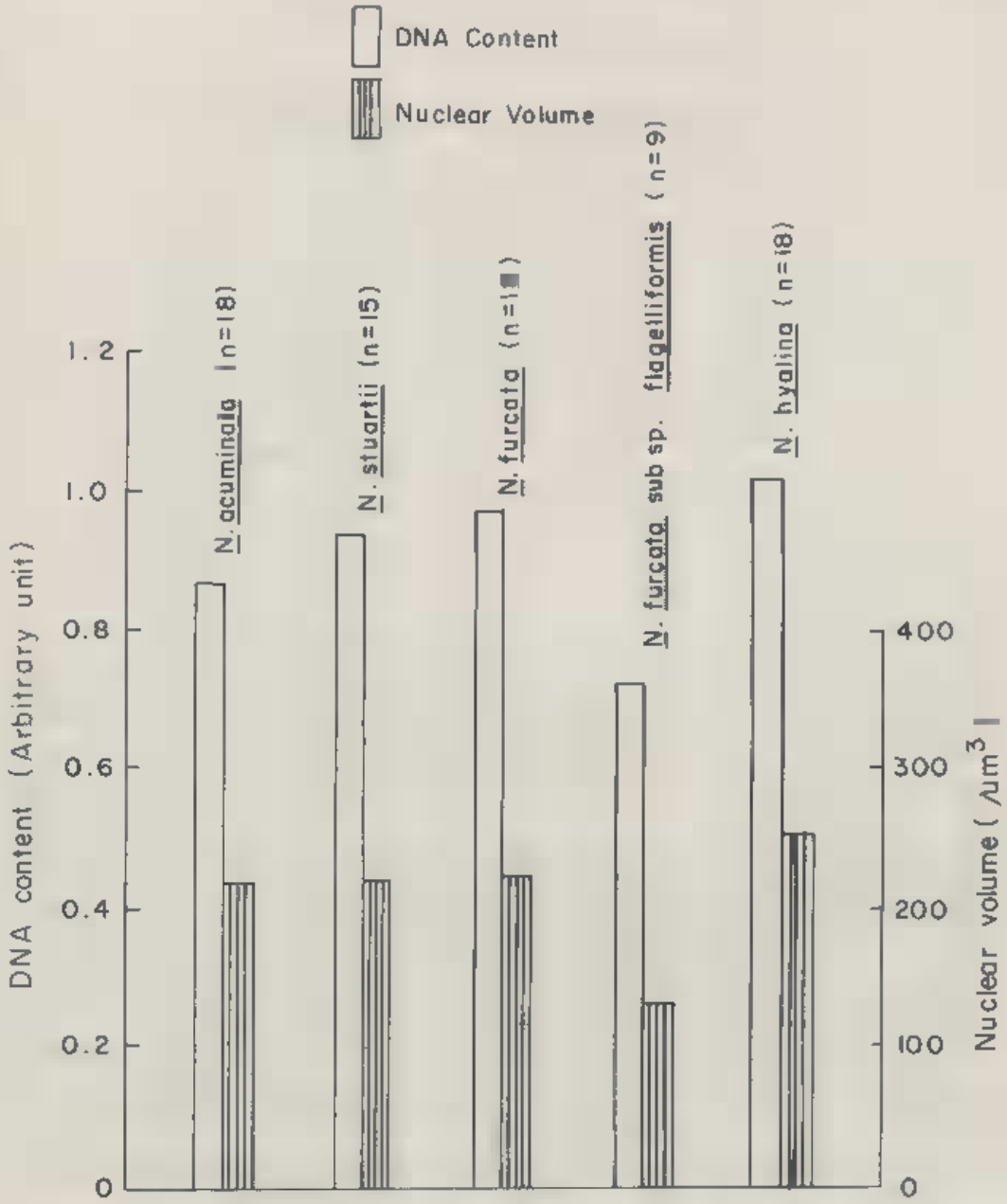

Fig. 1. – Histogram showing DNA content and nuclear volume in five species of *Nitella*.

some number was ■ = 9 and *N. acuminata* (n = 15), all other monoecious species studied had the same chromosome number of n = 18. Corresponding to the highest 2C DNA content, *N. hyalina* also showed the higher chromatin length and nuclear volume. The least amount of 2C DNA and nuclear volume was found in *N. furcata* subsp. *flagelliformis* having the lowest chromosome

Source : MNHN, Paris

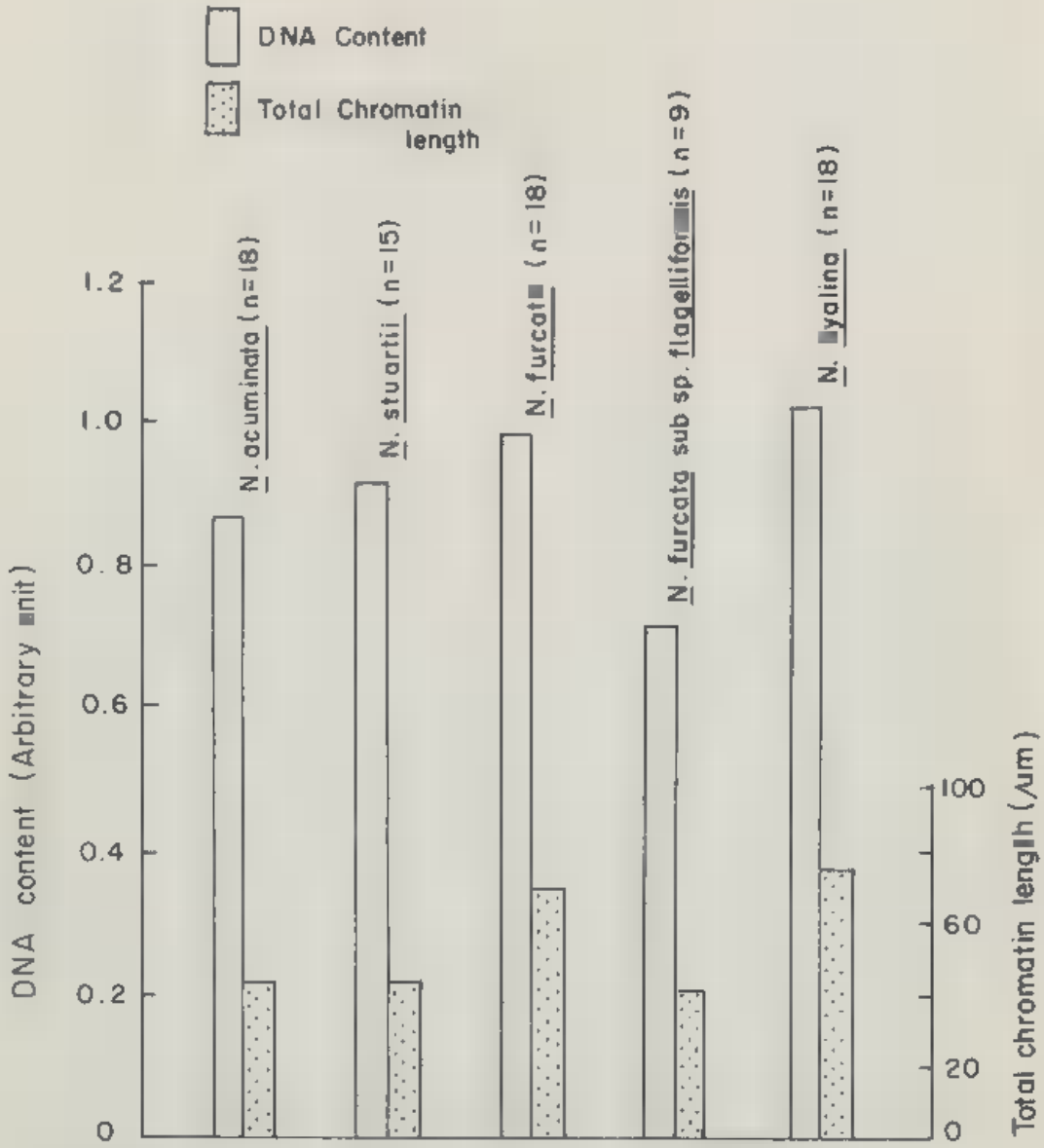

Fig. 2. — Histogram showing DNA content and total chromatin length in five species of *Nitella*.

number of n = 9. However, its chromatin length was slightly higher than *N. acuminata* and *N. stuartii* (n = 15) as the chromosomes are comparatively larger in size in this species than *N. acuminata* and *N. stuartii*. The nuclear volume of *N. furcata* subsp. *flagelliformis* was, however, the smallest found among all the *Nitella* species investigated here. Among the 18 chromosomed species of *Nitella*, *N. acuminata* showed the least amount of 2C DNA, nuclear volume and chromatin length, followed by an increase in all these parameters in *N. furcata* subsp.

Source : MNHN, Paris

Table I. — Showing chromosome number, total chromatin length, nuclear volume and DNA content (at 2C level) in five species of *Nitella*.

Name of the species	Chromosome number (n)	Total chromatin length (μm)	Nuclear volume (μm^3)	Amount of DNA in arbitrary unit (2C)
1. *Nitella hyalina* var. and f. *hyalina*	18	73.33	261.2	1.03 ± 0.001
2. *Nitella furcata* subsp. *furcata* var. and f. *furcata*	18	69.18	223.6	0.99 ± 0.006
3. *Nitella stuartii*	15	44.48	220.2	0.92 ± 0.002
4. *Nitella acuminata* var. and f. *acuminata*	18	44.60	210.5	0.87 ± 0.002
5. *Nitella furcata* subsp. *flagelliformis*	9	42.50	129.2	0.72 ± 0.002

var. and f. *furcata* and then *N. hyalina* where they were maximum. *N. stuartii*, another heteroclemous species investigated showed DNA content, nuclear volume and chromatin length intermediate between *N. acuminata* and *N. furcata* subsp. var. and f. *furcata*. Though variation in 2C value is clear among the five species, the variation is not much significant, which is 1.5 to 2 fold, in contrast to angiospermic genera.

Among the five species studied, *N. acuminata* and *N. stuartii* are arthrodactylous (dactyl 1 celled) and belong to the section *Rajia* and *Palia* respectively. Their DNA contents were also less, 0.87 ± 0.002 for *N. acuminata* and 0.92 ± 0.002 for *N. stuartii*. Under the Section *Tieffallenia*, *N. furcata* subsp. *furcata* and *N. furcata* subsp. *flagelliformis* have been studied. The DNA value of *N. furcata* subsp. *flagelliformis* was much less (0.72 ± 0.002), even less than *N. acuminata* and *N. stuartii*. Here the chromatin length and nuclear volume were also less. *N. furcata* subsp. *furcata* and *N. hyalina* (sect. - *Decandollea*) contained much more amount of DNA than the Arthrodactylous species. These were 0.99 ± 0.006 and 1.03 ± 0.001 unit respectively.

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Source : MNHN, Paris